Effects of Loading Density and Transport Water Volume on Ammonia Production, Stress, and Survival of Sacramento-San Joaquin Delta Fishes

Principal Investigators

Zachary A. Sutphin
Fisheries Biologist
Fisheries Application Research Group
Bureau of Reclamation
Denver, CO. 80225
zsutphin@usbr.gov

Donald E. Portz, Ph.D.
Fisheries Biologist
Fisheries Application Research Group
Bureau of Reclamation
Denver, CO. 80225
dportz@usbr.gov

Summary

Many resident and transient fish species to California's Sacramento-San Joaquin Delta (SSJD) have experienced precipitous declines in abundance over the last half century (Bennett and Moyle 1996, Moyle 2002, Brown and Moyle 2005). Though there are likely a multitude of factors that have contributed to the current state of the SSJD fishery (Moyle 2002), direct and indirect effects of southern SSJD water diversion facilities are commonly cited (Moyle and Williams 1989, Arthur *et al*. 1996, Brown *et al.* 1996). One particular indirect effect of SSJD water diversion facilities are stressors associated with transporting fish by truck from state and federal fish collection facilities, for subsequent release at central SSJD sites, as a means to prevent fish entrainment and pump induced mortality. Between 2000 and 2003 such operations resulted in the transportation of an average of nearly seven million fish per year (USBR 2009), including native species of concern, like endangered delta smelt (*Hypomesus transpacificus*) and Chinook salmon (*Onchorhynchus tschawyscha*), and ecologically important non-native Pelagic Organism Decline species, like threadfin shad (*Dorosoma petenense*) and striped bass (*Morone saxatilis*). Such operations may ultimately have population level effects on some species, and continued improvement of fish collection, handling (loading), transport, and release operations are a priority for both state and federal water resource agencies.

Fish transport from SSJD fish collection facilities consists of hauling fish in a closed (*e.g.*, no additional water provided throughout transport) cylindrical tank (1.2 m deep, 4.4 m long, mean volume post-transport = 6,455 L) that is provided continuous pure O_2 via oxygen diffusing airstones, over a maximum distance of 49.9 km (Sutphin and Wu 2008). To maintain fish health and maximize long-term survival, stressors that are common during such operations, including handling, confinement, unfavorable densities, and degraded water quality conditions, must be considered (Piper *et al.* 1982,

Berka 1986, Sutphin and Wu 2008). Maintenance of appropriate water quality conditions is often the limiting factor during fish transport, and is generally considered when developing fish transportation tables (Berka 1986, Emata 2000). In 2006 Reclamation biologists initiated a multi-phase research program to develop density and temperature dependent fish transportation tables, as a function of oxygen consumption and total ammonia nitrogen (TAN) production of SSJD fishes, for use at SSJD fish collection facilities. However, it is possible densities that permit appropriate water quality conditions are high enough to expose fish to physical (*i.e.,* abrasions and scale loss) and physiological stress (*i.e.,* crowding) that will effect short- and long-term survival. Density induced stress and associated conspecific interactions may also affect fish metabolism, resulting in increased oxygen consumption and ammonia (M_{TAN}) and carbon dioxide (M_{CO2}) production rates of transported fish. Transport truck oxygen production systems can generally be adjusted to meet the oxygen consumption demands of high densities of fish (B. Bridges 2009, personal communication). However, in closed (no additional water added) transport systems accumulated excretory products of fish can result in elevated levels of TAN, unionized ammonia, and carbon dioxide which can impair performance, health, and survival of fish (Meade 1985, Russo and Thurston 1991). Ammonia and carbon dioxide production may be exacerbated when fish are transported at high densities as a result of stress induced increases in metabolic rates. Measuring density dependent M_{CO2} and M_{TAN}, physiological stress and chronic (96-h) mortality of transported fish, paired with current research (water quality derived fish transportation tables), will provide information on methods for minimizing stress endured and maximizing acute and chronic fish survival during fish transportation operations from SSJD fish collection facilities.

In FY11 we constructed small-scale fish transportation containers, purchased equipment for the project, and initiated construction of our experimental set-up. We also completed a set of pilot studies during which we exposed juvenile rainbow trout (mean total length = 65.3–73.3 mm, mean wet weight = 4.9–6.3 grams) to varying densities (33–376 g/L) to evaluate fish transportation containers, our ability to adequately measure ammonia and CO_2 and provide O_2 to our fish at varying densities, and survival of salmon. During pilot research fish were exposed to a full water level (3.785 L), provided pure O_2 via an oxygen bubble diffuser (at varying pressures), and water quality (°C; DO, CO_2, pH, and TAN) were generally measured at 0, 20, 40, and 60 minutes post-insertion using a YSI pro-plus multi-parameter meter and CO_2 titration cells. After 60 min fish were removed from the transport container and put back into a 757-L holding tank. Results of our pilot research indicate ammonia production rates (MTAN) ranged from 0.003 mg/g/h (mg TAN/g of fish/h; 376 g of fish/L) to 0.007 mg/g/h (160 g/L). Though effects of density on fish stress were not measured during pilot studies, no fish mortalities were observed 72 h after testing. Also, the fish transport containers (design, volume, and construction) seem to work very well for our proposed research. We initially proposed to expose test fish to densities of 25, 50, 100, and 200 g of fish/L. Fish exposed to densities of 280.5 and 375.7 g/L during our pilot studies resulted in high ammonia (3.8 and 4.7 mg/L) and CO_2 levels (n/a and 70 mg/L), and lower pH levels (6.4 and 6.4) after 60 min. However, these fish incurred no mortality within 72 h. Based on our pilot results we are proposing to change our target test densities from 25, 50, 100, and 200 mg of fish/L to 25, 100, 200, and 300 g of fish/L. However, we request the TFCF's research team's input on this change.

Problem Statement

Fish transportation tables currently being considered for re-development by Reclamation biologists for use at south SSJD fish collection facilities are intended to provide fish diversion workers with the maximum temperature dependent density of fish that can be maintained for approximately 60−70 min to assure that unhealthy levels of ammonia (TAN and unionized ammonia), carbon dioxide, and oxygen are not reached. However, because transport operations from SSJD fish collection facilities are short in duration (<2 h) it is possible that densities recommended by the updated fish transportation tables may be high enough to expose fish to physical and physiological stress that will impair health and survival. Measuring density dependent M_{TAN}, physiological stress, and chronic (96-h) mortality, paired with current Reclamation research (water quality derived fish transportation tables), will provide information on methods for minimizing stress endured, and maximizing acute and chronic fish survival during fish transportation operations from SSJD fish collection facilities.

This proposal will be broken down into two different experimental designs, which will allow for management to take into consideration the necessity of this research and available FY12 budget, and decide the most appropriate means to determine the suitability of current fish transportation densities. **Option A** will allow us to quantify temperature and density dependent M_{TAN}, survival, and physiological stress on one species (threadfin shad). **Option B** will allow us to estimate the survival of three species of fish (Chinook salmon, striped bass, and threadfin shad) exposed to approximate density levels, as recommended by the current Bates Tables, and maximum water temperatures (species specific) they are likely to encounter during transport from the TFCF.

Goals and Hypotheses (Option A)

Goals:

1. Determine if additional physiological stress is caused by transporting fish at elevated densities (g of fish/L of water), and if there is an optimal density at which fish should be transported to minimize physiological stress.

2. Determine if transporting fish at varying densities affects post-transportation mortality (96 h) and if there is an optimal density at which fish should be transported to minimize post-transport mortality.

3. Determine if transporting fish at varying densities affects metabolic rates of fish, as a function of M_{CO2} and M_{TAN}, and if there is an optimal density at which fish should be transported to maintain appropriate M_{TAN} levels so TAN levels do not exceed 2 mg/L during transport.

Hypotheses (Null):

1. There will be no difference in blood constituent levels (Hct., glucose, lactate, cortisol) of fish exposed to densities of 25, 100, 200, and 300 g of fish/L of

water measured immediately after and 12 h post transport compared to basal levels (measured prior to transport).

2. There will be no difference in blood constituent levels (Hct., glucose, lactate, cortisol) between fish exposed to densities of 25, 100, 200, and 300 g of fish/L of water measured immediately after and 12 h post transport.

3. There will be no difference in acute (immediately following transport) and chronic survival (96 h post transport) between fish exposed to densities of 25, 100, 200, and 300 g of fish/L of water during transport.

4. There will be no difference in acute (immediately following transport) and chronic survival (96 h post transport) between fish exposed to 75 and 100% water volume during transport.

5. There will be no difference in M_{TAN} between fish exposed to densities of 25, 100, 200, and 300 g of fish/L.

Materials and Methods (Option A)

Source and Care of Fish

Threadfin shad were selected for this study because: (1) they are Pelagic Organism Decline species, (2) the two most abundant species salvaged at the SSJD fish collection facilities, and (3) are commonly present when high densities of fish are salvaged and transported at the facilities. A large quantity of test fish over three different test periods (as a function of three test temperatures) is required; therefore threadfin shad will be obtained from a commercial hatchery in Texas. Adult threadfin will be transported in 200-L transport tanks to Reclamations Technical Service Center and upon arrival will be maintained in continuously aerated 757-L circular flowthrough tanks. Water temperatures will be maintained at target temperature ± 0.5°C (where initial target temperature will be the temperature at which they were salvaged) and fish will be maintained under a natural photoperiod (37° 44' 23" N). When changes in water temperature are required, rate of change will be < 1.0°C/day. Fish will be fed an appropriate diet at 3–4% body weight per day.

Experimental Protocol: Effects of Elevated Densities on Stress and Survival During Transport.

We have selected 12 treatment conditions for testing: three water temperatures (9, 18, 27°C) and four densities (25, 100, 200, and 300 g of fish/L). Water temperatures encompass the approximate range threadfin shad are likely to encounter during transportation from the TFCF (Craft *et al*. 2008). Test densities are based on those measured by Sutphin and Wu (2008) during standard fish transportation operations from the TFCF (0.3–64.5 g/L), those recommended by preliminary water quality derived fish transportation tables data (100–175 g/L) to maintain TAN levels below 2 mg/L, current Bates Table recommendations (50–100 g/L) and densities that could potentially be achieved when large schools of fish are salvaged at the fish collection facilities (>200 g/L).

Prior to testing, fish will be randomly isolated as a function of treatment condition (density × transport container water volume) into 8 individual 190-L holding tanks, intended to simulate the TFCF fish haul-out bucket, and provided a unique mark using a fluorescent microsphere solution (New West Technologies, Santa Rosa, California.). This marking process will allow consolidation of all treatment fish during our post-transport survival assessment, but will also allow for an accurate estimate of loading density. Post marking, fish will be maintained at holding conditions for at least 7 days, during which mortality rates and feeding will be monitored to assure fish are healthy prior to experimentation. After the 7-day holding period two control fish will be removed from each holding tank, transferred to a bath containing a lethal dose of tricaine methansulfonate (MS-222; Argent Chemical Laboratories, Inc.; 200 mg/L), and sampled for blood according to methods outlined in Portz (2007), and a water sample will be collected to obtain water ammonia nitrogen level. Each 190-L tank will be lowered to a volume of 10-L and a partially filled (50%) 7.6-L fish transport bucket will be oriented directly below each holding tank drain. Each holding tank drain will be opened using a gate valve allowing water and fish to enter the fish transport buckets. Excess water will drain through a columnar screen at the top of the transport bucket.

Treatment fish will then be transported for 60 min on a flatbed truck. To simulate TFCF fish transportation operations, fish will be provided oxygen throughout transport and maintained above 7 mg/L, but no effort will be made to control other water quality parameters. After transport two treatment fish will be immediately removed from individual fish transport containers and bled in the identical manner as control fish. Simultaneously a water sample will be collected to obtain ammonia nitrogen level. After post-transport blood and ammonia samples are collected 20, 5, and 5 fish from each treatment (density × water volume) will be transferred to a 190-L holding tanks for 168-h survival assessment, a 50-L holding tank for 2-h post-transport stress-assessment, and a 50-L holding tank for 24-h post-transport stress assessment. Two-hour and 24-h post transport assessment will consist of bleeding fish (see Portz 2007), but given the amount of samples we will collect and cost for blood cortisol measurement we will only measure blood hematocrit, plasma lactate and glucose concentrations (see *Plasma Analysis* below).

Plasma Analysis

Blood samples will be immediately centrifuged for 4 min at 12,000 × g, effectively separating blood plasma from packed cells. Blood hematocrit (Hct.) levels for each individual sample will be recorded immediately and the plasma will be transferred to cryogenic freezing vials and stored in a liquid nitrogen dewar flask. Plasma lactate and glucose concentrations will be measured with a polarographic analyzer (YSI 2700 Select, Yellow Springs, Inc., Yellow Springs, Ohio) and plasma cortisol concentrations (for control and immediately post-transport fish only) will be measured by the University of California Davis Endocrinology Laboratory using a modified enzyme immunoassay.

168-h Mortality Analysis

After transport fish will be monitored every 24 h post transport through 168 h (7 d). Dead fish will be removed, identified by mark, and measured for length (fork, standard, and total lengths in mm) and wet weight (g). After 168 h is complete, all fish from each treatment will be measured for length and weight.

Ammonia Production Rates (M_{TAN})

Density dependent (group-mediated) M_{TAN} of fish will be estimated for each treatment condition using the following equation:

$$M_{TAN}\ (\text{mg/g/h}) = (TAN_{t0} - TAN_{t1}) \times V \times TW$$

Where TAN_{t1} is the TAN level (mg TAN/L) before fish are inserted into the transport tanks, TAN_{t0} is the TAN level after transport, V is the transport tank volume minus the volume of the fish transported, and TW is the total weight (g) of the transported fish.

Water Quality

Water temperature (°C), dissolved oxygen (mg/L), TAN (mg/L), carbon dioxide (CO_2, ppm), and pH levels will be measured before and after transport in each individual transport container, and daily in each holding tank.

Sample Size and Estimate of Time Required for Completion

A power calculation was carried out using post stress 96-h fish survival data from Hasan and Bart (2007) and post stress fish plasma constituent data from Portz (2007). Hasan and Bart (2007) assessed the effects of loading density (200, 300, and 400 g/L) and transport stress on mortality and physiological stress responses for rohu (*Labeo ronita*). Portz (2007) measured the effects of handling stress on plasma constituent levels of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Based on immediate and delayed mean mortality of transported fish at densities of 200, 300, and 400 g/L, as reported by Hasan and Bart (2007), and mean plasma constituent levels before and after 1 h transport and before and after handling stress as reported by Hasan and Bart (2007) and Portz (2007), respectively, we wanted to be able to detect a difference among means of 12 % (mortality) and 50 ng/mL (cortisol levels) using their reported standard deviations. Our desired power level is 0.90 and our alpha level is 0.05. We used SAS version 9.1, a statistical software package published by the SAS Institute Inc., to run the power calculation. Based on this calculation the minimum sample size needed to provide the desired power level, where sample size per group = n/2, is 12.

Because fish will be marked prior to testing, we can group fish as a function of treatment condition and all eight treatments will be combined in a single tank for 168-h survival assessment. It will require approximately 35 d to complete 12 replicates at each test temperature, and approximately 105 d to complete all data collection. Assuming adult threadfin shad weigh approximately 4.5 g, we transport fish at densities of 25, 100, 200, and 300 g/L and use 7.6-L transport buckets we will require approximately 861 marked fish per replicate and 10,332 shad per test temperature. Assuming we will use three test temperatures and account for 10% loss due to transport and holding mortality we will require a total of 34,096 threadfin shad. Because our current fish holding systems at the TSC cannot support this many fish we will pick up and transport fish on three different occasions.

Data Analysis

If assumptions necessary to model using parametric statistics (normality and equality of variance) are achieved, a two-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons will be used to test for differences between plasma constituent levels (Hct., lactate, glucose and cortisol), ammonia production rate and 1 68-h mortality levels for controls and water volume × fish density treatment combinations. If ANOVA assumptions are not met, Kruskal-Wallis ANOVA on ranks and Dunn's test will be employed. All statistical analyses will be conducted using Sigmastat 3.0 (Jandel Scientific, San Rafael, California) statistical software with an alpha level for all analyses set at 0.05.

Goals and Hypotheses (Option B)

Goals:

1. Measure percent survival, and effects on stress, fish metabolic rates (M_{TAN}, M_{CO2}), and water quality, of Chinook salmon, striped bass, and threadfin shad exposed to approximate densities as recommended by the TFCF Bates Tables and maximum water temperatures during transport from the TFCF

Hypotheses (Null):

1. Ninety-six hour survival of Chinook salmon, striped bass, and threadfin shad exposed to approximate densities as recommended by the TFCF Bates Tables and maximum water temperatures during transport from the TFCF will be >90%.

Materials and Methods (Option B)

Source and Care of Fish

Threadfin shad and striped bass were selected for this study because: (1) they are Pelagic Organism Decline species, (2) two abundantly salvaged species at the SSJD fish collection facilities, and (3) are commonly present when high densities of fish are salvaged and transported at the facilities. Chinook salmon were selected because (1) they were the species used to develop the initial Bates Tables, and (2) two of the ESU's in the SSJD are currently listed as either threatened (Central Valley Spring-Run) or endangered (Sacramento River winter-run) by federal regulatory agencies. All test species will be obtained from commercial or state/federal fish hatcheries across the western United States. Fish will be transported in 200-L transport tanks to Reclamations Technical Service Center and upon arrival will be maintained in continuously aerated 757-L circular flowthrough tanks. Water temperatures will be maintained at target temperature ± 0.5°C (where initial target temperature will be the temperature at which they were salvaged) and fish will be maintained under a natural photoperiod (37° 44' 23" N). When changes in water temperature are required, rate of change will be <1.0°C/day. Fish will be fed an appropriate diet at 3–4% body weight per day.

Experimental Protocol: Effects of Elevated Densities on Stress and Survival During Transport

For experimental Option B, we are proposing to test all three species at one density, as recommended by the current TFCF fish hauling tables and the approximate maximum temperature at which they would likely be exposed to during transport from the TFCF. Density is generally reported as weight per unit volume (*i.e.,* g/L). However, the Bates Tables are temperature dependent and designed by size class, and reported as thousands of fish that can be transported (as a function of maximum fish load). We assumed our test fish would likely be in the 1.5–3.0 in Bates Table category, and that salmon, shad, and striped bass in this length bin would have a weight of 4.5 g (based on length vs. weight relationships developed from fish collected at the TFCF). We were able to estimate the density of fish for testing by making these assumptions and knowing that the truck volume Bates used during experimentation was 3,795 L. Threadfin shad will be tested at 27°C and a density of 42 g/L (~9 fish/replicate), striped bass will be tested at 24°C and a density of 60 g/L (~13 fish/replicate), and Chinook salmon will be tested at 18°C and a density of 52 g/L (~12 fish/replicate). All other methods (fish tagging, transportation, and measuring stress indices, survival, metabolism, and water quality) will follow the protocols as outlined in Option A.

Sample Size and Estimate of Time Required for Completion

For experimental Option B, we will not be testing our three test species at similar temperatures or density levels; therefore, we will not be making comparisons across treatment conditions. However, our methods will allow us to estimate effects of current recommended maximum temperature-dependent fish transport density levels on metabolic rates, physiological stress, and survival of Chinook salmon, threadfin shad, and striped bass. If Option B is selected, we will employ a sample size of n = 12 (as described for Option A). Assuming one treatment conditions per species (1 size × 1 temperature × 1 density) and a sample size of n = 12, we will require approximately 250 fish per species for testing. Because we have only one temperature control unit for holding and testing fish, water temperature will be the limiting factor for testing and we will only be able to test one species at a time.

Coordination and Collaboration

Experimental design and research updates will be provided at requested Tracy Technical Advisory Team (TTAT) and/or Central Valley Fish Facilities Review Team (CVFFRT) meetings. However, primary coordination and collaboration will be between TFCF staff and biologists, the Fisheries and Wildlife Resources Group, and the interagency TTAT.

Endangered Species Concerns

Hatchery fish will be used for testing.

Dissemination of Results

Research updates will be provided and/or presented at regularly scheduled TTAT and CVFFRT meetings. The primary deliverables will be a Tracy Volume Series Report, as well as a publication in a peer-reviewed scientific journal. However, posters and/or oral presentations will also be given at appropriate scientific meetings (*i.e.*, American Fisheries Society). Additionally, information obtained in this study will be used in the implementation of new fish transportation tables for use at south SSJD fish collection facilities.

Literature Cited

Arthur, J. F., M. D. Ball, and S. Y. Baughman. 1996. *Summary of federal and state water project environmental impacts in the San Francisco Bay-Delta estuary, California*. Pages 445–495 *in* J. T. Hollibaugh, editor. San Francisco Bay: The Ecosystem, Further Investigations into the Natural History of San Francisco Bay and Delta with Reference to the Influence of Man. Pacific Division of the American Association for the Advancement of Science, San Francisco, California.

Bennett, W. A. and P. B. Moyle. 1996. *Where have all the fishes gone? Interactive factors producing fish declines in the Sacramento-San Joaquin Estuary*. San Francisco Bay: the ecosystem 1:519–542.

Berka, R. 1986. *The transport of live fish: a review*. United Nations, Rome.

Bridges, B. 2009. Bureau of Reclamation, Tracy Fish Collection Facility, Byron, California, personal communication.

Brown, L. R. and P. B. Moyle. 2005. *Native fish communities of the Sacramento-San Joaquin watershed, California: a history of decline*. Pages 75–98 *in* F. Rinne, R. Hughes, and R. Calamusso, editors. Fish Communities of Large Rivers of the United States. American Fisheries Society, Bethesda, Maryland.

Brown, R., S. Greene, P. Coulston, and S. Barrow. 1996. *An evaluation of the effectiveness of fish salvage operations at the intake of California aqueduct, 1979–1993*. Pages 497–518 *in* J. T. Hollibaugh, editor. San Francisco Bay: The Ecosystem. Pacific Division, American Association for the Advancement of Science, San Francisco, California.

Craft, D., M. Del Hoyo, R. Housewright, and J. Fields. 2008. *Semi-continuous water quality measurements at the Tracy Fish Collection Facility*: 7-Year Summary.

Emata, A.C. 2000. *Live transport of pond-reared Milkfish, Chanos chanos, broodstock*. Journal of the World Aquaculture Society 31(2):279–282.

Hasan, M. and A.N. Bart. 2007. *Effects of capture, loading density and transport stress on the mortality, physiological responses, bacterial density and growth of rohu Labeo rohita fingerlings*. Fish Physiology and Biochemistry 33:241–248.

Meade, James W. 1985. *Allowable ammonia for fish culture*. Progressive Fish Culturist 47:135–145.

Moyle, P. B. 2002. Inland Fishes of California. University of California Press, Berkeley.

Moyle, P. B. and J. E. Williams. 1989. *Biodiversity loss in the temperate zone: decline of the native fish fauna of California*. Conservation Biology 4:275–284.

Piper, R. G. and coauthors. 1982. *Fish hatchery management*. Department of the Interior, U.S. Fish and Wildlife Service, Washington, D.C.

Portz, D.E. 2007. *Fish-holding-associated stress in Sacramento River Chinook salmon (Oncorhynchus tshawytscha) at south Delta fish salvage operations: effects on plasma constituents, swimming performance, and predator avoidance*. Doctoral dissertation. University of California, Davis.

Russo, R.C. and R.V. Thurston. 1991. *Toxicity of ammonia, nitrite, and nitrate to fishes*. Pages 58–89 *in* D.E. Brune and J.R. Tomasso, editors. Aquaculture and Water Quality, World Aquaculture Society, Baton Rouge, Louisiana.

U.S. Bureau of Reclamation (USBR). 2009. The Tracy Research Website: Tracy Fish Facility Improvement Program.

Sutphin, Z.A. and B.J. Wu. 2008. Changes in water quality during fish-hauling operations at the Tracy Fish Collection Facility.